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RESEARCH ARTICLE

Influence of Different Extraction Solvents and the Micronutrient Composition on the Bioactive Properties and Antimicrobial Efficacy of *Spirulina Maxima* Extracts

T Sowmya Priyadharshini, Rengasamy Sathya*

Abstract

Spirulina is a highly valued, nutrient-rich, blue-green alga and is recognized for its high protein content, as well as for its functional food components such as vitamins, amino acids, minerals, and antioxidants, with potential benefits for immune health. The growing recognition of Spirulina's nutritional and health-promoting properties has contributed to its rising demand as a functional food ingredient. The present study aimed to evaluate the influence of different extraction solvents and the micronutrient composition on the bioactive properties and antimicrobial efficacy of *Spirulina maxima* extracts. In the current study, *S. maxima* was successively extracted based on solvent polarity, and each extract was identified by TLC analysis. The micronutrient content of the powdered sample was determined using an Atomic Absorption Spectrophotometer (AAS). Fe (2.43 mg/g) and Cu (0.72 mg/g) were the predominant micronutrients, while Ni and Cd were not detected in the raw powdered sample. High-Performance Liquid Chromatography (HPLC) was used to identify and estimate Phycocyanin. The antimicrobial activity for both the extract and phycocyanin against Gram-positive and Gram-negative bacteria — *Pseudomonas aeruginosa* (ATCC 15692), *Staphylococcus aureus* (ATCC 23235), *Bacillus subtilis* (ATCC 15245), *Klebsiella pneumoniae* (ATCC 700603), and *Escherichia coli* (ATCC 25922) — was evaluated using the agar diffusion method, and the results were compared with those of the standard Gentamicin (50 µg/mL). The serial dilution method was used to determine the Minimum Inhibitory Concentration (MIC) of the *S. maxima* extract, using Gentamicin as the positive control. The ethanolic extract exhibited the highest activity, and the MIC values ranged from 16.8 to 146.7 µg/mL. The findings highlight *S. maxima* as a promising natural source of bioactive compounds, where solvent polarity and micronutrient composition significantly contribute to its antimicrobial potential.

Keywords: Agar diffusion, Antimicrobial, Extracts, MIC, Solvent.

Introduction

Human health is greatly impacted by the worldwide problem of food safety. Food-related infections affect more than 2 billion people annually, contributing to an increase in food-borne epidemics worldwide (Uyttendaele *et al*., 2016). Food safety and public health agencies must regulate pathogenic microorganisms to effectively prevent and control food-borne diseases. The food sector frequently uses chemical preservatives or antibiotics to control food deterioration and harmful bacteria (Harwood *et al*., 2018). The adverse effects of synthetic preservatives and antibiotics have led to a significant increase in regulatory restrictions. However, the need to create substitute substances to fight food-spoiling pathogens has grown as a result of the short effective life duration of antimicrobials and the emergence of resistance microorganisms (Silva and Lidon, 2016). Natural sources of organic food preservatives with strong antibacterial and antioxidant properties include secondary metabolites such as polyphenols (Manso *et al*., 2021). With the increasing prevalence of bacteria that are resistant to antibiotics and more tolerant of food processing and preservation methods, the use of plant-derived secondary metabolites in food preservation has gained significant attention. In 2017, the World Health Organization (WHO) published a list of twelve bacterial families identified as global priority pathogens due to their significant threat to human health. Gram-negative

Department of Microbiology, PRIST Deemed To Be University, Vallam, Thanjavur, Tamil Nadu 613 403, India.

***Corresponding Author:** Rengasamy Sathya, Department of Microbiology, PRIST Deemed To Be University, Vallam, Thanjavur, Tamil Nadu 613 403, India, E-Mail: sathyaram1984@gmail.com

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bacteria are mostly targeted as they can pass on genetic information to other bacteria, thus spreading drug resistance (Asokan *et al*., 2019). The food industry is showing a growing preference for natural, food-grade preservatives to improve food safety and quality, since synthetic and chemical preservatives have been linked to negative health impacts (Pedan *et al*., 2019).

Spirulina is filamentous cyanobacteria that float freely and grows organically in warm water. It is mostly grown in ponds and lakes, which are its natural habitat, all over the world. Since the early sixteenth century, it has been consumed directly by Asian, African, and Mexican communities. Spirulina represents the dried biomass of the oxygenic, photosynthetic cyanobacterium *S. maxima*, which occurs naturally in both freshwater and marine habitats worldwide. An essential part of the human diet, this alga has been utilized as a source of protein and vitamin supplements without side effects. Spirulina is currently included on the list of substances classified as *generally recognized as safe* (GRAS) by the US Food and Drug Administration (Belay, 2002). Additionally, it includes minerals, particularly iron, and vitamins, mainly B12 and provitamin A (β-carotenes). Additionally, it is abundant in tocopherols, γ-linolenic acid, and phenolic acids (Karkos *et al*., 2011). Apart from that it also contains high amount of G-linolenic acid, stearidonic acid, eicosapentaenoic acid, docohexanoic acid etc. (Soheili *et al*., 2011). With the presence of different constituents, it shows versatile pharmacological and therapeutic activities viz. anti-inflammatory efficacy through inhibiting histamine release from mast cell (Karkos *et al*., 2011). It has potent anti-inflammatory (Muga *et al*., 2014), antioxidant (Tobón-Velasco *et al*., 2013), antidiabetic (Deng and Chow, 2010), antibacterial (Sarada *et al*., 2011), and antiviral (Hernández-Corona *et al*., 2002) efficacies with the presence of omega-3 fatty acids. It also improves immunity and lowers blood pressure and cholesterol. Traditionally it also alleviates versatile disease conditions viz. diabetes, cancer, heart disease, arthritis, and even depression (Soni *et al*., 2017). Certain aspects of the activity of *S. maxima* species remain poorly understood. To the best of our knowledge, the influence of micronutrients and solvents on the bioactive components, antimicrobial activity, and their correlations in *S. maxima* has not been thoroughly reported, forming the basis for the present study (Pianta *et al*., 2025; Bougatef *et al*., 2025; Ilieva *et al*., 2024).

Materials and Methods

Collection and Authentication of Spirulina

S. maxima sample was collected from CFTRI (Central Food Technological Research Institute, Mysore-90 (Latitude: 12° 19′ 1.68″ N Longitude: 76° 38′ 8.28″ E) and was authenticated by Scientist-F of CFTRI. The sample was preserved for the future reference (CFTRI/SM-1203/2024-25) in Department of Microbiology, Mallige College of Pharmacy, Bangalore.

Determination of micronutrients

Atomic Absorption Spectrophotometer (AAnalyst 200, PerkinElmer, Switzerland) was used to determine the concentration of various micronutrients in the powdered *S. maxima* sample. Acid digestion was performed using a mixture of concentrated sulfuric acid and perchloric acid in a 4:2 ratio. The sample was heated gradually to approximately 200 °C until dense white fumes appeared and a clear, white residue was obtained, indicating complete digestion. The elemental analysis of the digested samples was carried out after dilution with deionized water, and the final volume was made up to 50 mL in a volumetric flask. Specific analytical wavelength, for example, zinc (214 nm), iron (372 nm), and copper (327 nm), were used for flame atomization, with an air-acetylene flame serving as the oxidizing medium. A reagent blank was used as the reference, and the elemental concentrations were expressed in mg/g on a dry weight basis. All analyses were performed in triplicate (Das and Tribedi, 2015).

Extraction of *S. maxima* sample

S. maxima raw powder sample (2 kg) was successively extracted by Soxhlet method for 16 hrs using various solvents namely, n-hexane, chloroform, ethyl acetate, ethanol and water, finally yield was calculated. Further, phycocyanin was extracted from the extracted crude *S. maxima* sample. Approximately 15 g of the biomass was homogenized in 1.5 L of 100 mM phosphate buffer (pH 7.0) using an agitated extraction system, maintaining a 1:100 (w/v) ratio. The suspension was stirred at ambient temperature for 3–4 hours, followed by centrifugation to separate cellular residues. The obtained supernatant (crude extract) was then purified stepwise through microfiltration membranes with pore sizes of 1.0 µm and 0.2 µm, and subsequently through ultrafiltration using a 50 kDa molecular weight cut-off (MWCO) membrane. The final extract yield was quantified, and its composition was verified by HPLC analysis.

TLC of the extracts and phycocyanin

Thin-layer chromatography (TLC) was carried out for the crude extracts obtained using different solvents, along with phycocyanin. Approximately 50 µL of each crude fraction was applied onto the TLC plate, and the separation was achieved using a hexane–ethyl acetate solvent system (7:3, v/v). The developed spots were visualized by spraying the plate with p-anisaldehyde reagent. The eluted plates were dried completely and the chromatograms were visualized under UV at long and short UV 365 nm 254 nm and the movement of the phytochemical along with solvent was measured (Rf value).

HPLC analysis

Phycocyanin was estimated using HPLC method. Methanol and ammonium acetate 3% (7:3, v/v) were utilised as the

mobile phase in a C-18 column (250 x 4.6 mm). The detection wavelength was 620 nm with the temperature fixed at 25 °C. 15 µL injection volume with 1 ml flow rate was maintained (Izadia and Fazilat, 2018).

Microorganism used for the experimentation

Gram positive organisms namely, *Staphylococcus aureus* (ATCC 23235), *Bacillus subtilis* (ATCC 15245), and Gram-negative organism viz. *Pseudomonas aeruginosa* (ATCC 15692), *Klebsiella pneumonia* (ATCC 700603), *Escherichia coli* (ATCC 25922) were used for present study. The bacterial isolates used in this study were procured from the Department of Microbiology, Bangalore University, Bangalore, India. The strains were maintained through routine sub-culturing on nutrient agar plates at the Department of Microbiology, Mallige College of Pharmacy, Bangalore. A stock solution of Gentamicin (50 µg/mL) was prepared in sterile distilled water following the method described by Das *et al*. (2009), and 0.1 mL of this solution was employed for the antimicrobial evaluation.

Determination of Minimum Inhibitory Concentration (MIC)

Mueller-Hinton broth (HiMedia Labs, India) was used as the inoculation medium for colony formation from 24-hour-old bacterial cultures. An aliquot of 1.0 mL from the most effective extract (200 µg/mL) was combined with 1 mL of nutrient broth to prepare a serial dilution series of 150, 100, 50, 25, and 12.5 µg/mL for the samples collected from different zones (Atata *et al*., 2003). Subsequently, 0.1 mL of each dilution was transferred into 9 mL of nutrient broth containing the standardized bacterial inoculum, adjusted to a 0.5 McFarland turbidity standard ($\approx 1.0 \times 10^8$ CFU/mL). The cultures were incubated at 37 °C for 24 hours, and bacterial growth was assessed visually. The MIC values obtained were then used to select appropriate concentrations for further antimicrobial activity testing.

Antimicrobial Assay

The antibacterial activity of all extracts was evaluated using the agar well diffusion method (Das *et al*., 2012). Each bacterial strain was revived and maintained on its specific culture medium, followed by incubation at 35–37 °C for 24–25 hours. The extract solutions (100 mg/mL) were sterilized through membrane filtration before being used in the assay. Using a sterile cork borer, 6 mm wells were made in the inoculated agar plates, and 50 µL of each extract was added to each well. Gentamicin discs (50 µg/disc) were used as positive controls, and the inoculated plates were incubated at 37 °C for 16 hours to evaluate antimicrobial efficacy. The antibacterial activity was determined by measuring the diameter of the inhibition zones (mm) using a standard ruler. Each experiment was performed in triplicate to ensure reproducibility and minimize experimental error (Kaur *et al*., 2013).

Correlation study

Micronutrient content was correlated with the yield of the extract and with the antimicrobial efficacy. Thereafter, extract was correlated with the antimicrobial activity.

Statistical analysis

The micronutrient content was analyzed based on the mean values obtained from three independent replicates. In addition, all microbicidal activity data were presented as the mean ± standard error of the mean (SEM), and differences were considered statistically significant at $P < 0.05$ and $P < 0.01$. The data were tabulated and graphically illustrated using Microsoft Excel and GraphPad Prism version 5, respectively.

Results

Micronutrient content

Atomic Absorption Spectrophotometer was used for determination of micronutrient content in the raw powder of *S. maxima*. The results indicated that the contents of Fe, Cu, and Zn were higher than those of the other micronutrients (Table 1). Furthermore, the micronutrient content was also determined for all the different extracts of the *S. maxima* sample, and the results were summarized in Table 2. It showed Fe, Cu and Zn content were higher with the ethanol extract of *S. maxima* extract.

Yield of the extract

S. maxima powder sample was extracted with successive solvent, based on the polarity and revealed the higher yield with ethanol extract (278.24 g) followed by aqueous extract (247.23 g), ethyl acetate extract (179.22 g), chloroform extract (73.81 g) and hexane extract (63.87 g). The final yield was calculated and depicted in Figure 1.

Figure-1 showed the per cent content of ethanol extract was higher than others. The ethanolic extract showed the highest yield (13.91%), followed by the aqueous extract (12.36%). Thereafter, the phycocyanin content was obtained at 2.34 g from 15 g of the *S. maxima* raw powder.

TLC study

Furthermore, all the extracts along with phycocyanin was separated and identified by TLC method using precoated silica gel plate with hexane and ethyl acetate (7:3 v/v) mobile phase for the extracts and methanol and ammonium acetate 3%, in the ratio of 7:3, v/v was used for the identification of phycocyanin. Result revealed ethanol extract gave more separation and identification of multiple compounds available in the same than the other extracts. Interestingly, in all the extracts phycocyanin was identified when compared with the standard C-phycocyanin (Rf = 0.38) as shown in Table 2 and Figure 2 (a - d). Spots were visualized by spraying with p- anisaldehyde. The eluted plates were dried completely and the chromatograms were visualized

Table 1: Micronutrient content in *S. maxima* powder sample

Micro-nutrients	*Wavelength (nm)*	*Content (mg/g)*	*Content (mg/g) of extract*				
		Powdered sample of S. maxima	*Hexane extract*	*Chloroform extract*	*Ethyl acetate extract*	*Ethanol extract*	*Water extract*
Fe	372	2.43 ± 0.03	1.03 ± 0.23	1.00 ± 0.21	1.14 ± 0.26	2.60 ± 0.32	1.32 ± 0.81
Mn	279.5	0.23 ± 0.10	0.11 ± 0.16	0.08 ± 0.10	0.18 ± 0.10	0.26 ± 0.44	0.19 ± 0.33
Cu	327	0.72 ± 0.11	0.21 ± 0.34	0.20 ± 0.14	0.26 ± 0.30	0.75 ± 0.06	0.46 ± 0.22
Zn	214	0.28 ± 0.41	0.28 ± 0.41	0.21 ± 0.21	0.23 ± 0.44	0.31 ± 0.02	0.26 ± 0.10
Se	196	0.51 ± 0.20	0.24 ± 0.33	0.20 ± 0.11	0.29 ± 0.30	0.64 ± 0.03	0.33 ± 0.10
Mo	386	0.14 ± 0.21	0.08 ± 0.53	0.04 ± 0.12	0.06 ± 0.22	0.18 ± 0.44	0.08 ± 0.13
Ni	232	ND	ND	ND	ND	ND	ND
Cd	229	ND	ND	ND	ND	ND	ND
Co	241	0.07 ± 0.11	ND	ND	0.04 ± 0.23	0.08 ± 0.22	0.02 ± 0.14

Values were mean ± SEM (n =3)

Table 2: Detection of the spots based on Rf values

Sl. No.	*Mobile Phase*	*Extracts*	*Spots*	*Rf Value*
1.	Hexane: Ethyl acetate 3:7	Hexane	10	0.08,0.17,0.52,0.63,0.69,0.76,0.86,0.91,0.95,1.0.
		Chloroform	10	0.08,0.19,0.5,0.63,0.69,0.76,0.86,0.91,0.93,0.97.
		Ethyl Acetate	10	0.04,0.08,0.1,0.23,0.45,0.58,0.65,0.73,0.78,0.86.
		Ethanol	11	0.04,0.08,0.1,0.23,0.34,0.45,0.56,0.73,0.82,0.91,0.95.
		Water	02	0.02,0.08.
2.	Methanol: 3% ammonium acetate	Ethanol	07	0.04,0.16,0.38, 0.56, 0.74, 0.86, 0.91
		c-Phycocyanin	01	0.38

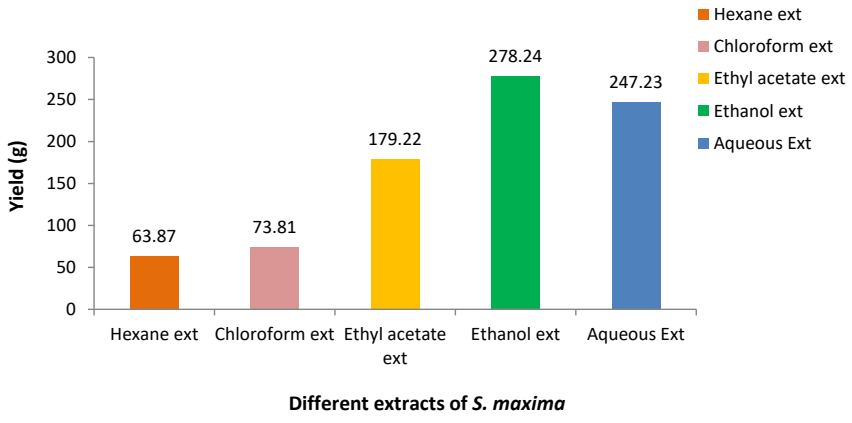


Figure 1: Yield of the extracts of *S. maxima* powder

under UV at long and short UV 366 nm and 254 nm and the movement of the phytochemical along with solvent was measured with Rf value.

HPLC study

Identified phycocyanin was further confirmed with HPLC study. The result revealed that standard C-phycocyanin was eluted at 13.526 min and also detected in ethanol extract of *S. maxima* at Rt of 13.502 min (Figure 3 and 4).

Based on the various analytical parameters and chromatographic techniques ethanol *S. maxima* extract was used for further antimicrobial activity along with extracted phycocyanin.

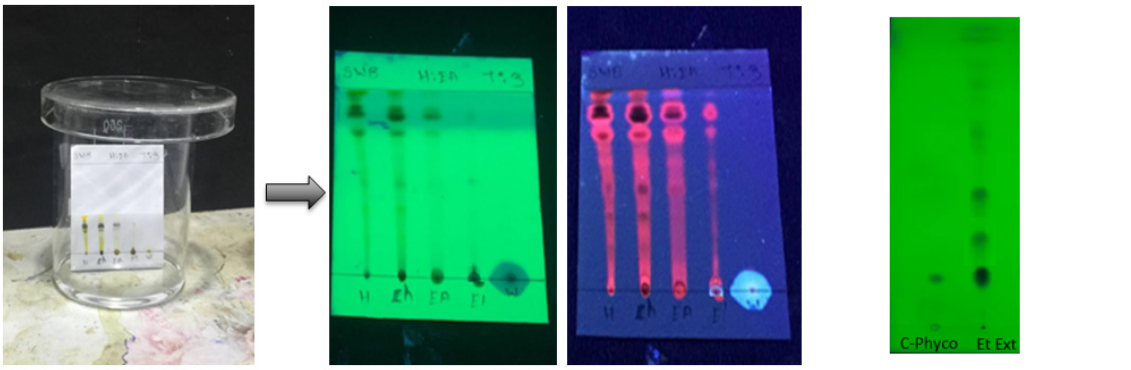


Figure 2(a-d): TLC of various *S. maxima* extracts and detection of phycocyanin in ethanol extract

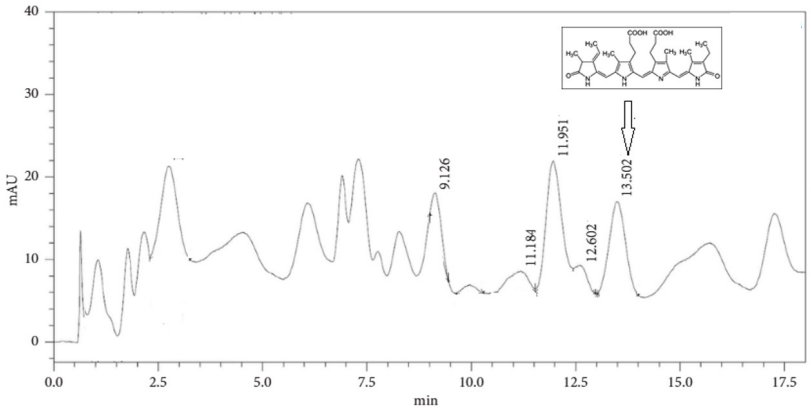


Figure 3: Presence of Phycocyanin in *S. maxima* ethanol extract

Determination of MIC

The results demonstrated that the ethanolic extract of *S. maxima* exhibited MIC values ranging from 16.7 to 146.7 µg/mL against all tested microbial strains (Figure 5). Based on the MIC values, further dose were selected for the antimicrobial study. The MIC values of the *S. maxima* ethanolic extract (16.7–146.7 µg/mL) were higher than those of standard Gentamicin (0.25–4.0 µg/mL against common Gram-positive and Gram-negative bacteria) as reported by Barnes *et al*. (2023), Kadeřábková *et al*. (2024), and Hemeg *et al*. (2020).

In-vitro Antimicrobial study

Finally, the antimicrobial screening of the ethanolic extract of S. maxima and phycocyanin was performed in triplicate using the agar well diffusion assay. Standardized broth cultures of test bacterial isolates (*S. aureus; B. subtilis; P. aeruginosa; K. pneumonia;* and *E. coli*) were used as causative organisms. Result revealed *S. maxima* ethanol extract showed higher activity against Gram positive organisms than Gram negative and the activity was concentration dependent. The same trend was followed by phycocyanin (Table 3).

Table 3 showed higher activity against *B. subtilis* (13.78 mm) followed by *S. aureus* (13.43 mm) by *S. maxima* ethanol extract at 200 µg/mL when compared with Standard Gentamicin against *B. subtilis* (17.50 mm) followed by *S. aureus* (16.46 mm). Similarly, phycocyanin also showed dose dependent activity when compared with the activity against Gram positive and Gram-negative organisms. Both the extracts were active against both the causative organisms but showed better result against Gram positive organisms.

Correlation study

Micronutrient content was correlated with yield of the extract and showed positive correlation (Table 4).

Table 4 revealed that all the selected micronutrients showed positive correlation with the extracts. Among them Fe content was significantly correlated with ethanol and aqueous extracts ($p<0.05$). All other extract also showed satisfactory and positive correlation, with the aqueous extract displaying the strongest association. Interestingly,

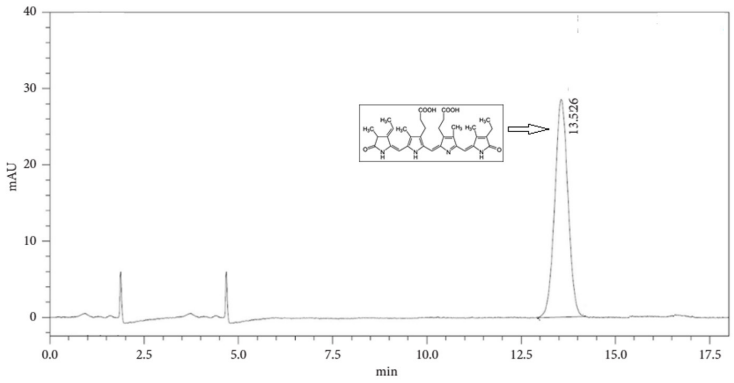
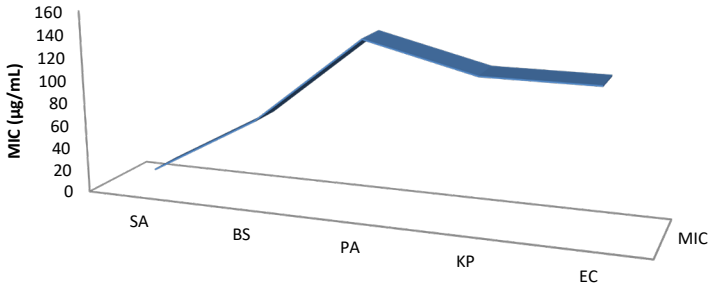


Figure 4: HPLC of Standard C-Phycocyanin



	SA	BS	PA	KP	EC
MIC	16.7	69.47	146.7	123.45	123.48

SA = *S. aureus*; BS = *B. subtilis*; PA = *P. aeruginosa*; KP = *K. pneumonia* EC = *E. coli*;

Figure 5: MIC of *S. maxima* ethanol extract against various microorganisms

ethanol extract was also positively correlated with the aqueous extract. Figure 6 shows that the ethanolic extract of *S. maxima* exhibited a strong positive correlation between extract yield and Fe content ($R^2 = 0.994$).

Table-5 showed correlation study among micronutrient content with antimicrobial activity. It revealed that all micronutrient content showed only positive correlation with the antimicrobial activity. Figure 7 illustrates a significant positive correlation between the Fe content and the antibacterial activity against *B. subtilis* ($R^2 = 0.9098$). Interestingly, it also showed that zone of inhibition among the organisms were significant.

Phycocyanin showed dose-dependent activity against the tested microorganisms. The inhibition zone was highest against *B. subtilis* (13.31 mm), followed by *S. aureus* (13.01 mm), showing similar activity to the *S. maxima* crude extract (Table 3).

Discussion

The role of micronutrients in various activities is on high demand in the present era. They not only provide the nutrients in the plant body but also provides degree of immunity against pests, bacteria etc. Hence the present study was carried out to understand the role and the impact of micronutrients available in the *S. maxima* species against microbial pathogens. Micronutrient content was determined with Atomic Absorption Spectrophotometer in *S. maxima* species and showed high content in extracts than powdered *S. maxima*. This may be due to solubility of the micronutrients in the solvents than powdered form and also improves the activation of the plant enzymes which further improves growth and development. Earlier literature also showed similar effect (Deli *et al*., 2020). Various mineral compositions of *Spirulina platensis* have been determined using inductively coupled plasma optical emission spectrometry (Liestianty *et al*., 2019). However, comprehensive reports on the micronutrient content of *S. maxima* — including Fe, Mn, Cu, Zn, Mo, Ni, Cd, and Co — are limited. In this study, an Atomic Absorption Spectrophotometer (AAS) was employed for precise quantification of the elemental composition.

Various solvents were used for the extraction of *S. maxima* powder, and among them, ethanol produced the highest yield. This can be attributed to the relatively high dielectric constant and polarity of ethanol, which allows it

Table 3: Antimicrobial activity of the *S. maxima* ethanol extract and phycocyanin

Parameters	*Conc (µg/mL)*	*SA*	*BS*	*PA*	*EC*	*KP*
S. maxima ethanol ext	50	8.45 ± 0.07	9.06 ± 0.21	ND	8.70 ± 0.33	ND
	100	9.76 ± 0.32	10.93 ± 0.06	9.02 ± 0.20	9.75 ± 0.42	9.02 ± 0.32
	150	11.89 ± 0.11	11.43 ± 0.07	9.98 ± 0.21	10.06 ± 0.11	9.78 ± 0.11
	200	13.43 ± 0.34	13.78 ± 0.33	10.34 ± 0.30	10.87 ± 0.07	10.46 ± 0.60
Phycocyanin	50	9.67 ± 0.21	8.98 ± 0.32	7.08 ± 0.20	8.34 ± 0.07	ND
	100	10.61 ± 0.34	10.02 ± 0.11	7.99 ± 0.11	9.05 ± 0.83	9.45 ± 0.33
	150	12.09 ± 0.43	11.54 ± 0.21	9.59 ± 0.41	10.04 ± 0.32	10.43 ± 0.08
	200	13.01 ± 0.26	13.31 ± 0.34	10.28 ± 0.27	10.58 ± 0.10	10.08 ± 0.32
Std	50	16.46 ± 0.22	17.50 ± 0.54	15.43 ± 0.33	13.44	13.07

SA = *S. aureus*; BS = *B. subtilis*; PA = *P. aeruginosa*; EC = *E. coli*; KP = *K. pneumonia*

Table 4: Correlation coefficient study among the micronutrients (MN) with higher yield of the different extracts

Micro Nutrients	*Fe*	*Mn*	*Cu*	*Zn*	*Se*	*EtOH Ext*	*Aq Ext*	*Ethyl Ext*
Fe	1							
Mn	-0.583	1						
Cu	-0.962	0.658	1					
Zn	-0.977	0.763	0.977	1				
Se	-0.780	-0.039	0.718	0.646	1			
EtOH Ext	0.994**	0.754	0.988*	0.974	0.601	1		
Aq Ext	0.980*	0.068	0.566	0.972	0.447	0.828	1	
Ethyl Ext	0.973	0.364	0.439	0.675	0.127	-0.960	-0.674	1

*p<0.05; **p<0.01 (statistically significant); EtOH Ext- Ethanol extract; Aq Ext = Aqueous extract; Ethyl Ext – Ethyl acetate extract

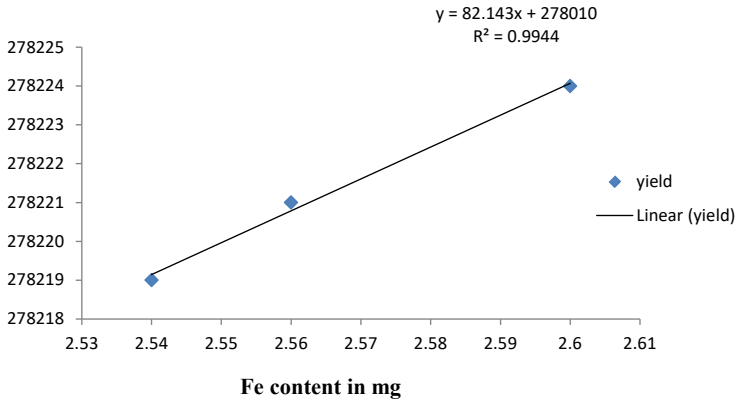


Figure 6: Correlation study of high yielded *S. maxima* ethanol extract with high content of Fe

to dissolve a wide range of bioactive compounds, including both polar and moderately non-polar constituents (Das *et al*., 2023). Therefore, ethanol has the capability to dissolve many active constituents. The similar effect was resulted with the *S. maxima* species. Thereafter, protein complex-phycocyanin was extracted and result showed good yield in aqueous solvent. Phycocyanin is very important protein complex which is blue in colour and has versatile therapeutic applications viz. anticancer, anti-inflammatory, antioxidant etc. Due to protein in nature, it is highly soluble in aqueous solvent rather alcohol and hence the extraction was carried out from other species of spirulina i.e. *S. platensis* (Izadia and Fazilat, 2018; Nikolova *et al*., 2024). There are limited reports on the extraction of phycocyanin from *S. maxima* species; therefore, in this study, extraction was performed using an aqueous solvent following a similar approach.

Table 5: Correlation coefficient among micronutrient content with antimicrobial activity

MN	Fe	Mn	Cu	Zn	Se	BS	SA	PA	EC	KP
Fe	1									
Mn	-0.583	1								
Cu	-0.962	0.538	1							
Zn	-0.977	0.956	0.839	1						
Se	0.090	-0.478	0.059	-0.037	1					
BS	0.909	0.329	0.402	0.553	0.892	1				
SA	0.878	0.310	0.411	0.431	0.782	0.551	1			
PA	0.848	0.031	0.028	0.234	0.382	0.718	0.976	1		
EC	0.460	0.029	0.309	0.276	0.409	0.582	0.996**	0.983*	1	
KP	0.801	0.207	0.310	0.319	0.531	0.700	0.986*	0.992**	0.984*	1

*p<0.05; **p<0.01 (statistically significant); BS= *B. subtilis*; SA= *S. aureus*; PA= *P. aeruginosa*; EC= *E. coli*; KP = *K. pneumonia*

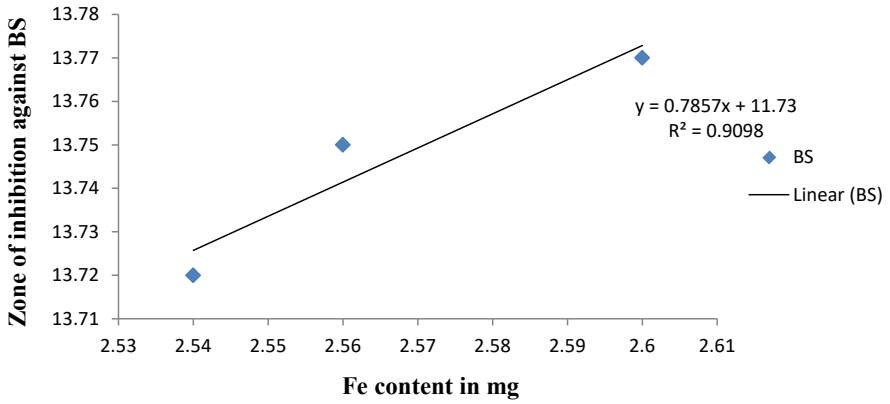


Figure 7: Correlation study among Fe content with higher zone of inhibition against BS

The constituents were identified and separated through TLC and HPLC methods. TLC is an affinity-based separation technique where extract can be identified and separated based on the affinity towards mobile phase. Many previous studies also carried out the same technique for the similar activities (Kagan and Flythe, 2014). Furthermore, phycocyanin was confirmed with the HPLC analysis. Many earlier reports supported the present data (Izadia and Fazilat, 2018).

Finally, antimicrobial efficacy was determined based on the MIC results. MIC reflects the minimum concentration of an antimicrobial agent that inhibits the growth of particular pathogenic organism. In the present study, serial dilution method was used for the determination of MIC against the selected microorganisms. Earlier literature also reported the importance of MIC determination for the antimicrobial study (Barnes*et al*., 2023; Kadeřábková *et al*., 2024). Further, Gram positive and Gram-negative organisms were selected for the antimicrobial study and result was compared with broad spectrum antibiotics based on the determination of zone of inhibition diameter. Various concentration of *S. maxima* ethanol extract was used for the study along with phycocyanin and resulted dose dependent activity for both the samples. Earlier studies have also reported a similar effect, demonstrating concentration-dependent antimicrobial activity (Hemeg *et al*., 2020; Vaou *et al*., 2021). In the study, the micronutrients play an immense role. Iron (Fe) plays an important role in microbial growth regulation. Under certain conditions, it can exert bacteriostatic effects that depend on both its concentration and the surrounding pH. Solutions containing ferric ions (Fe^{3+}) may inhibit bacterial proliferation when reduced to ferrous ions (Fe^{2+}), due to the generation of reactive oxygen species (ROS) that damage cellular components. This may explain why the extract form of *S. maxima* showed stronger antimicrobial activity than the purified phycocyanin (Sun *et al*., 2011). Zinc (Zn) is one more essential micronutrient that exhibits potent antimicrobial activity in the form of Zn^{2+} in aqueous solution (Pasquet *et al*., 2014). Selenium (Se) is an important micronutrient which helps in generating reactive oxygen species (ROS). It damages microbial cell components like lipids, proteins, and DNA, which finally causes cell death of bacterial pathogens (Cremonini *et al*., 2016). Manganese (Mn) in the soluble form of MnO_2 shows potent antimicrobial

activity as with the same mechanism of selenium (Friães *et al*., 2023). Likewise other micronutrients are also helped in combating the microbial pathogens.

Further, the correlation study was carried out for the potential relationship between the various factors and the activity. Various statistical data among the yield, micronutrient content and antimicrobial activity were established for the correlation study. This study contributed to the scientific validation of the research hypothesis by demonstrating the potential therapeutic activity, particularly the antimicrobial efficacy, of *S. maxima* extracts.

Conclusion

Overall, the study concluded that ethanol extract of *S. maxima* showed superior among all other extracts in terms of yield, and content of micronutrients. Various micronutrient content was determined by Atomic Absorption Spectrophotometer and showed their potential role in antimicrobial efficacy when the correlation coefficient study was performed. The positive correlation among the yield and the micronutrients content with antimicrobial activity were observed. Further, Phycocyanin was extracted and performed antimicrobial activity along with the ethanol extract of *S. maxima* species and result showed both were active against Gram positive and Gram-negative organism which concluded that *S. maxima* species and phycocyanin both are the drug of choice as broad-spectrum antimicrobial efficacy. To the best of our knowledge, this is the first report on effect of micronutrients in antimicrobial activity of *S. maxima* species.

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